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ORIGINAL PAPER

Synthesis of new benzo[*f*]chromene-based heterocycles targeting anti-proliferative activity

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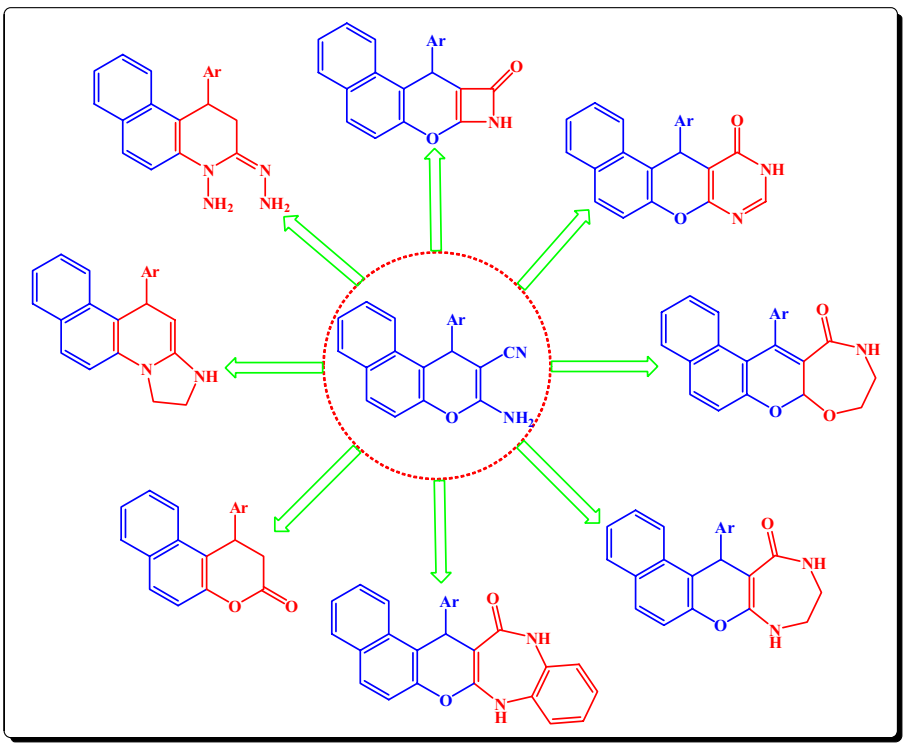
Abstract

In this article *β*-enaminonitrile **1** undergoes intramolecular cyclocondensation reaction under acidic conditions to generate novel chromeno[2,3-b]azet-9-one derivative **2** in good yield. Ring expansion of the four membered ring of the azet-2(1H)-one derivative **2** to six and/or seven membered rings was achieved via reaction of compound **2** with different nitrogen nucleophiles. A new series of benzochromeneone, benzochromenopyrimidine, and benzo[*f*]coumarin derivatives were prepared via reaction of *β*-enaminonitrile **1** with different C-electrophiles. The structures of the products have been affirmed on the basis of analytical and spectral data. The anti-proliferative activity of the newly synthesized compounds against two human epithelial cell lines; liver (HepG2) and breast (MCF-7) in addition to normal fibroblasts (WI-38) was investigated. Derivatives **4** and **10** had significant and selective anti-proliferative activity against liver and breast cancer cell lines without harming normal fibroblasts.

Graphic abstract



Extended author information available on the last page of the article

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Keywords Benzo[*f*]chromene · Chromeno[2,3-b]azet-9-one · Benzo[*f*]chromeno[2,3-d]pyrimidines · Benzo[*f*]chromeneone · Benzo[*f*]coumarin · Intramolecular cyclocondensation · Anti-proliferative activity

Introduction

Benzochromenes and fused chromenes are significantly established molecules that display diverse biological activities, like antileishmanial [1], antibacterial, antifungal [2–4], anti-proliferative agents [5], hypolipidemic [6], blood platelet antiaggregating [7], vascular-disrupting [8], antioxidant [9, 10] effects and activities. Furthermore, benzochromenes were considered as an emboldening skeleton for the development of potent antitumor agents. For example, *β*-enaminonitrile/esters **(A)** [11–14] are classified as effective antiproliferative agents, while *β*-enaminonitriles **(B)** [15] have efficacious apoptotic and cytotoxic behaviors against different cell lines: MCF-7, HepG-2, MDA-MB-231, T-47D, KB, PC3, and SK-N-MC. Additionally, *β*-enaminonitriles **(C)** represent antiproliferative and c-Src kinase inhibitory activities [16], as shown in Fig. 1.

Moreover, benzochromenopyrimidines exhibit antitumor activities. For example, the methylimino compounds **(D)** [13, 17], amino–imino compounds **(E)** [13, 14, 17, 18], and the amino–imino compounds **(F)** [13, 14, 17] have higher considerable strong anticancer activities against MCF-7, HepG-2, and HCT-116, in comparison to the different standard drugs: Vinblastine, Colchicine, and Doxorubicin, as presented in Fig. 2.

These promising results encouraged us to more expand our research toward the synthesis of heterocyclic systems of prospect biological applications. In continuation of our former research [19], we synthesize here some more analogs of benzo[*f*]chromene moiety as a target unit with anti-proliferative evaluation against two of human cancer cell lines.

Results and discussion

As a part of our concern in the synthesis of a wide domain of heterocyclic systems with biological applications [20–33], our present research is interested with the utility of 3-amino-1-(2-chlorophenyl)-1*H*-benzo[*f*]chromene-2-carbonitrile **(1)** [19] in the design and synthesis of new series of benzo[*f*]chromene-based heterocycles and investigate their anticancer activities. Noteworthy the synthesis of benzochromenes fused with azetidinones was not reported. Hence, in this study, we report the synthesis of analog novel derivative of chromeno[2,3-b]azet-9-one by intramolecular cyclocondensation reaction of *β*-enaminonitrile under acidic conditions. Thus, refluxing the *β*-enaminonitrile **1** with formic acid afforded the unexpected product 10-(2-chlorophenyl)-8,10-dihydro-9H-benzo[5,6]chromeno[2,3-b]azet-9-one **(2)** rather than the corresponding fused pyrimidinone derivative **3** [18, 34, 35] (Scheme 1).

A close analysis of the IR spectrum of the azet-2(1H)-one derivative **2** revealed the presence of absorption bands for NH group at 3439 cm^{-1}, CO group at 1776 cm^{-1} with

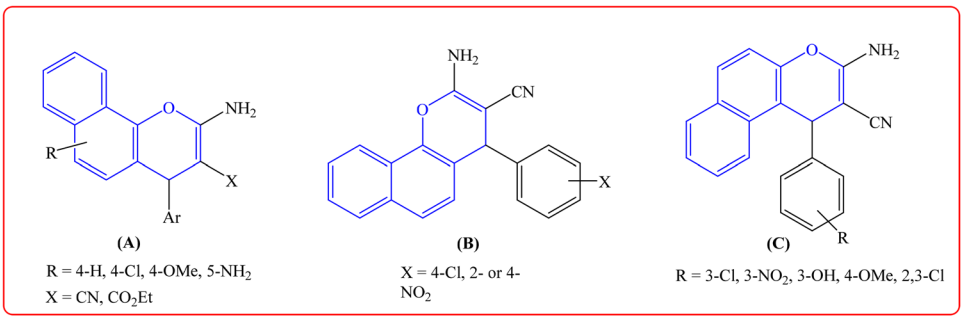


Fig. 1 Structures of fused chromene derivatives with biological activities

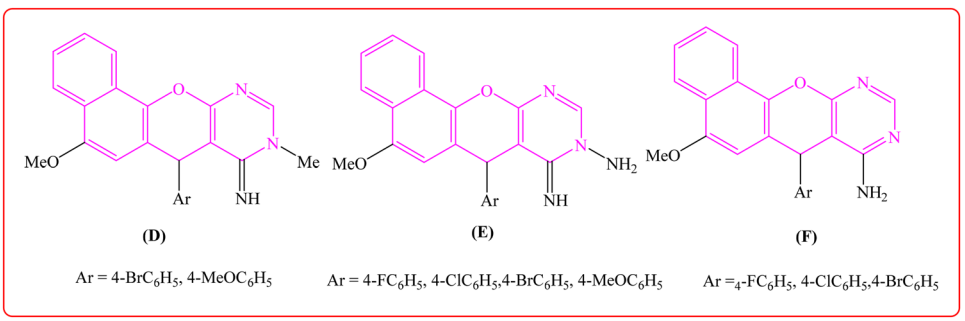
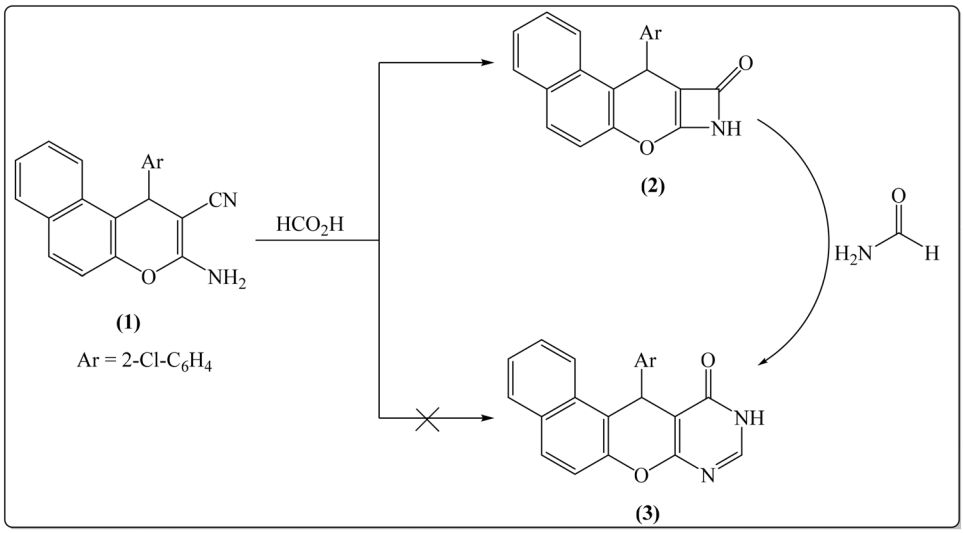


Fig. 2 Structures of some benzochromenopyrimidines with apoptotic and cytotoxic effects

Scheme 1 Synthesis of novel chromeno[2,3-b]azet-9-one derivative **2**



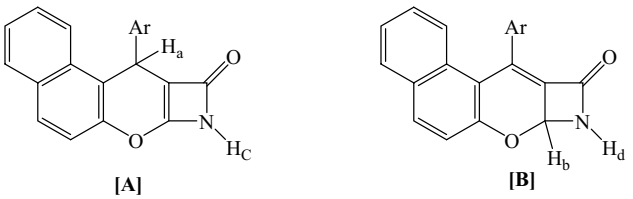


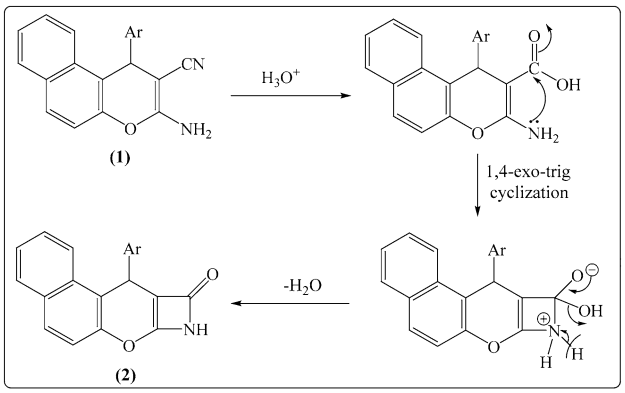
Fig. 3 1,3-proton shift of azet-2(1*H*)-one derivative **2**

the disappearance of absorption band for C≡N group. The ^{1}H NMR ($CDCl_3$) spectrum of **2** strongly supported the assigned structure as it showed that in solution, compound **2** exists in two forms **[A]** and **[B]** (cf. Fig. 3) in ratio 1:1 due to 1,3-proton shift. H_a and NH_c appear as two singlets at δ 5.71 ppm and 4.26 ppm, while H_b and NH_d appear as two doublets at δ 5.96 ppm ($J = 6.6$ Hz) and 4.42 ppm ($J = 6.9$ Hz) and both singlet and doublet for NH_c and NH_d are exchangeable in D_2O. In addition, the aromatic protons appear as multiplet at δ 6.68–8.03 ppm.

The ambiguity was overcome through the ^{13}C NMR ($CDCl_3$) spectrum which agrees with the assigned structure as there are two values for CO group at 159.9 and 158.5, C_4-pyran has two values at 39.84 (in A) and 133.38 (in B), C_2-pyran has also two values at 149.7 (in A) and 77.36 (in B). The formation of compound **2** can be explained by the hydrolysis of the CN-group of benzo[*f*]chromene-2-carbonitrile **1** to form the corresponding carboxylic acid intermediate which underwent intramolecular cyclocondensation with loss of water molecule, as illustrated in Scheme 2.

On the other hand, heating azet-2(1H)-one derivative **2** with formamide under reflux afforded the pyrimidinone derivative **3** through ring expansion (Scheme 1).

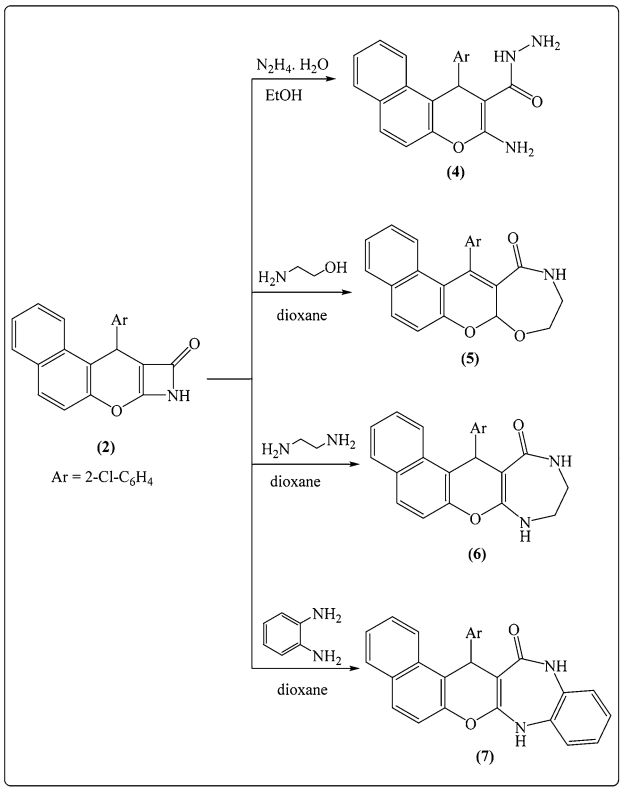
The behavior of the azet-2(1H)-one derivative **2** toward different nitrogen nucleophiles was investigated. Thus,



Scheme 2 The suggested mechanism for formation of the title compound **2**

hydrazinolysis of compound **2** with hydrazine hydrate in ethanol yielded the corresponding benzo[*f*]chromene-2-carbohydrazide **4** which was formed through nitrogen nucleophilic attack upon the electrophilic carbonyl carbon atom of CO-group of the azet-2(1H)-one derivative **2**, followed by ring opening (Scheme 3). The IR spectrum of compound **4** revealed the presence of strong absorption bands at 3442, 3389, 3324 cm^{-1} for (NH, NH_2) and 1650 cm^{-1} for (CO). The low value of carbonyl stretching frequency may be attributed to its conjugation with C=C of pyran ring in addition to intermolecular hydrogen bond with NH_2-group. In the meantime, the ^{1}H NMR spectrum of compound **4** displayed the following signals: 5.90 (s, 1H, HC_4-pyran), 6.32 (s, 2H, NH_2, exchangeable by D_2O), 6.90–8.32 (m, 10H, Ar–H), 9.25 (br.s, 2H, NH_2, exchangeable by D_2O), 11.16 (br.s, 1H, NH, exchangeable by D_2O).

Ring expansion of the four membered ring of the azet-2(1H)-one derivative **2** to seven membered ring was achieved via reaction of compound **2** with bidentate nucleophiles such



Scheme 3 Reaction of the azet-2(1H)-one derivative **2** with hydrazine hydrate and bidentate nucleophiles

as ethanolamine, ethylenediamine and/or *o*-phenylenediamine to yield the corresponding benzochromeno[3,2-f][1,4] oxazepinone derivative **5** and benzochromeno[3,2-e][1,4] diazepinone derivatives **6** and **7**, respectively (Scheme 3). The formation of compounds **5–7** was assumed to take place via nucleophilic attack by nitrogen nucleophile of the bidentate nucleophile upon CO-group of **2**, followed by cyclization with elimination of ammonia molecule.

Spectroscopic data of compounds **5–7** were in agreement with their assigned structures for this reaction. The IR spectra exhibited absorption bands corresponding to NH group in the range 3360–3137 cm^{-1} as well as bands for C=O group at 1684–1724 cm^{-1}. The ^{1}H NMR spectra of compounds **5**,**6** showed that the methylene groups (O$\underline{CH_2}$ and NH$\underline{CH_2}$) of compound **5** appear as triplet signals at δ 4.59 and 4.50 ppm, respectively, while the methylene groups (2 NH$\underline{CH_2}$) of compound **6** appear as multiplet signals at δ 3.48–3.61 ppm. Also, ^{1}H NMR spectra of compounds **5–7** exhibited two singlets integrated for one proton in the range δ 5.92–5.49 ppm for H_a and H_b (due to 1,3-H shift, cf. Fig. 3) in addition to other signals attributed to NH and aromatic protons.

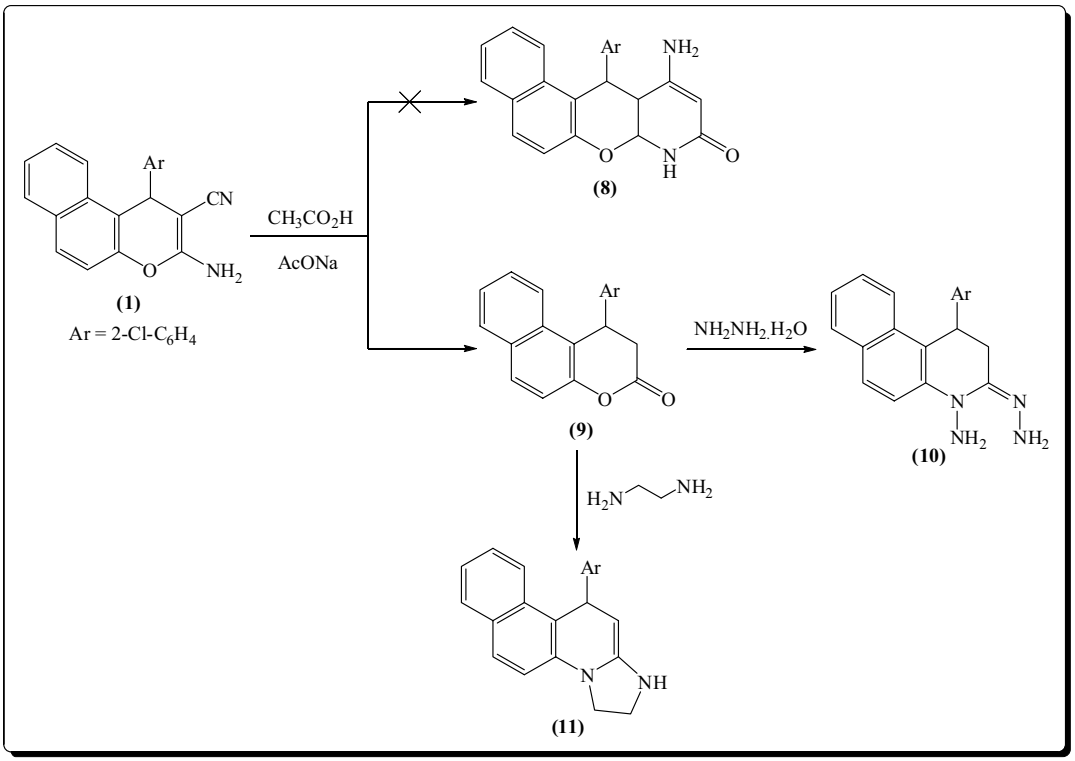
It has been reported that [36] treatment of β-enaminonitrile with glacial acetic acid in presence of fused sodium acetate furnish the fused pyridinone **8**. Herein, the behavior of the understudy β-enaminonitrile **1** toward acetic acid in the presence of fused sodium acetate was investigated and afforded unexpected product that was identified as 1-(2-chlorophenyl)-1,2-dihydro-3*H*-benzo[*f*]chromen-3-one **(9)** (Scheme 4). The benzo[f]chromeneone **9** was previously prepared via one-pot three-component condensation of Meldrum's acid with benzaldehyde and β-naphthol [37].

The structure of benzo[*f*]chromeneone **9** was deduced on the basis of its elemental and spectroscopic data. Thus, IR spectrum of **9** displayed absorption band at 1769 cm^{-1} attributed to existence of CO of cyclic lactone ring with the absence of any bands for NH, NH_2 or C≡N groups. In addition to, the ^{1}H NMR ($CDCl_3$) spectrum showed the following signals at δ (ppm): 3.20 (d, 2H, CH_2, J = 6.0 Hz), 5.45 (t, 1H, C4-pyran, J = 3.0 Hz), 6.71–7.92 (m, 10H, Ar–H). Moreover, the mass spectrum of **9** revealed the existence of correct molecular ion peak at m/z = 308 (M^+, 11%), in addition to base peak at m/z = 231.

An acceptable mechanism of formation of benzo[*f*] chromeneone **9** could be depicted in Scheme 5.

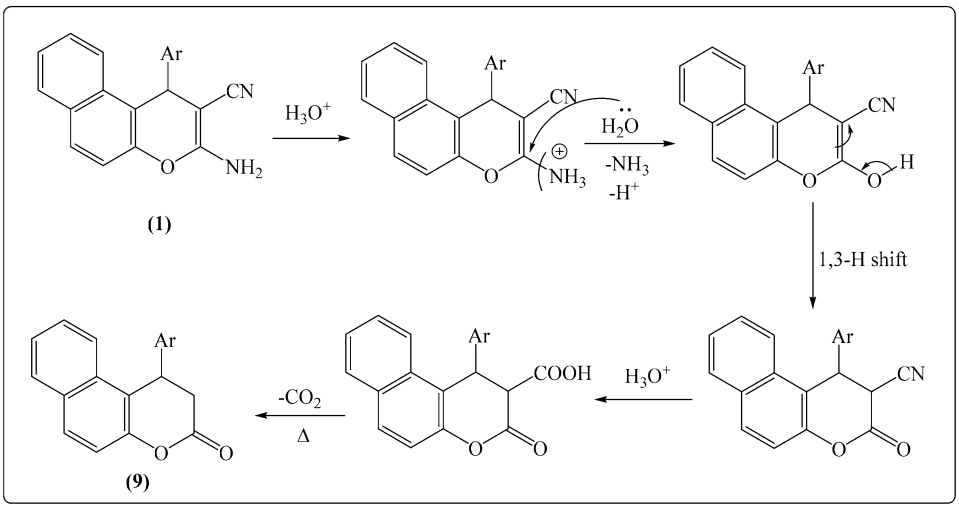
An interesting heterocyclic ring transformation from benzo[*f*]chromenone to benzo[*f*]quinoline was achieved via reaction of the benzo[*f*]chromenone **9** with hydrazine hydrate to afford benzo[*f*]quinoline derivative **10** (Scheme 4). The existence of ν_{NH2} at 3327, 3280 cm^{-1} besides $\nu_{C=N}$ at 1646 cm^{-1} in the IR spectrum of compound **10** with the disappearance of stretching frequency of CO group in addition to the appearance of two broad singlet signals of $2NH_2$ protons exchangeable with D_2O in the ^{1}H NMR spectrum reinforces the assigned structure **10**. On the other hand, when benzo[*f*]chromeneone **9** and ethylenediamine were heated under reflux in dioxane, benzo[*f*]imidazo[1,2-a]quinoline derivative **11** was obtained (Scheme 4).

The β-enaminonitrile **1** proved to be a valuable synthon for the synthesis of a diversity of pyrimidine derivatives. Subsequently, treatment of β-enaminonitrile **1** with formamide at reflux yielded the desired aminopyrimidine derivative **12** (Scheme 6). Supporting chemical evidence for compound **12** was forthcoming from treatment of **12** with freshly distilled acetic anhydride which gave the corresponding diacetyl derivative **13** as sole product. The structure of **13** was confirmed form its IR spectrum which displayed two absorption bands at 1729 and 1704 cm^{-1} representing 2CO of diacetyl group with the disappearance of absorption bands of NH_2-functionality. Acylation of β-enaminonitrile **1** with freshly distilled acetic anhydride led to a mixture of diacetyl derivative **14** and pyrimidinone derivative **15** which were separated by fractional crystallization in yield 75%, and 20%, respectively (Scheme 6). The pyrimidinone derivative **15** was previously obtained via an intramolecular Pinner reaction of β-enaminonitrile **1** with acetic acid in the presence of phosphoryl chloride ($POCl_3$), followed by a Dimroth rearrangement [38]. However, when the reaction



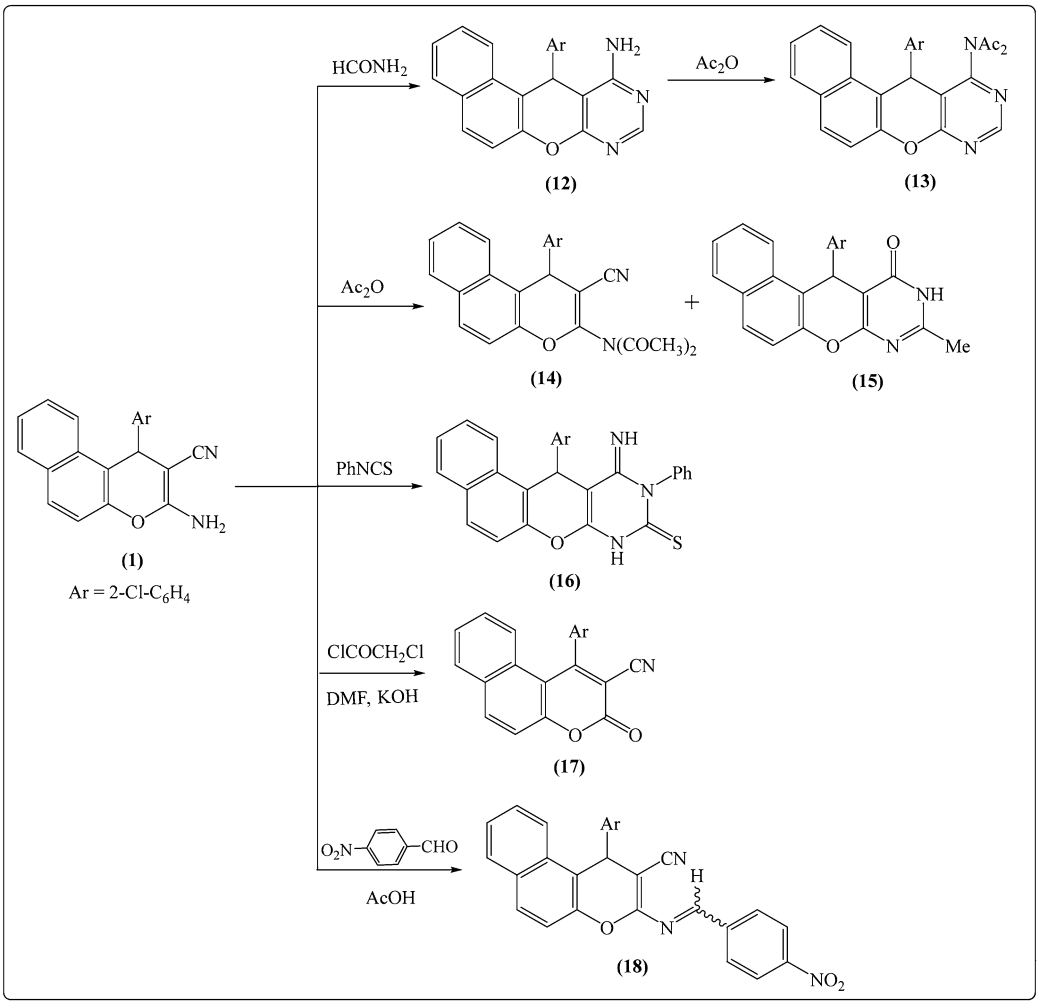
Scheme 4 The synthetic course of compounds **9**–**11**

Scheme 5 A plausible mechanism for conversion of **1** to **9**



was conducted in presence of dry pyridine, the yield of pyrimidinone derivative **15** increased to 84%. It is evident that presence of pyridine as a base promoted the cyclization of previously formed monoacetyl derivative to pyrimidinone derivative **15** rather than further acetylation to give diacetyl derivative **14**.

When enaminonitrile **1** allowed to react with phenyl isothiocyanate in neat conditions, it yielded the benzochromeno[2,3-d]pyrimidine-9-thione derivative **16** (Scheme 6). The disappearance of νC≡N in the IR spectrum of **16** and the presence of νNH at 3450, 3388 cm^{-1} and νC=N at 1606 cm^{-1} supported the suggested structure. ^{1}H NMR spectrum ($CDCl_3$) of **16** gave further evidence as it disclosed the presence of two singlet each integrated for one proton at 1.60 and 6.22 ppm which were exchanged with D_2O indicating the existence of two NH groups in addition to singlet signal of H-benzylic at 6.52 ppm and a multiple signal at 7.02–7.84 ppm for 15 aromatic protons.



Scheme 6 The synthetic course of compounds **12**–**18**

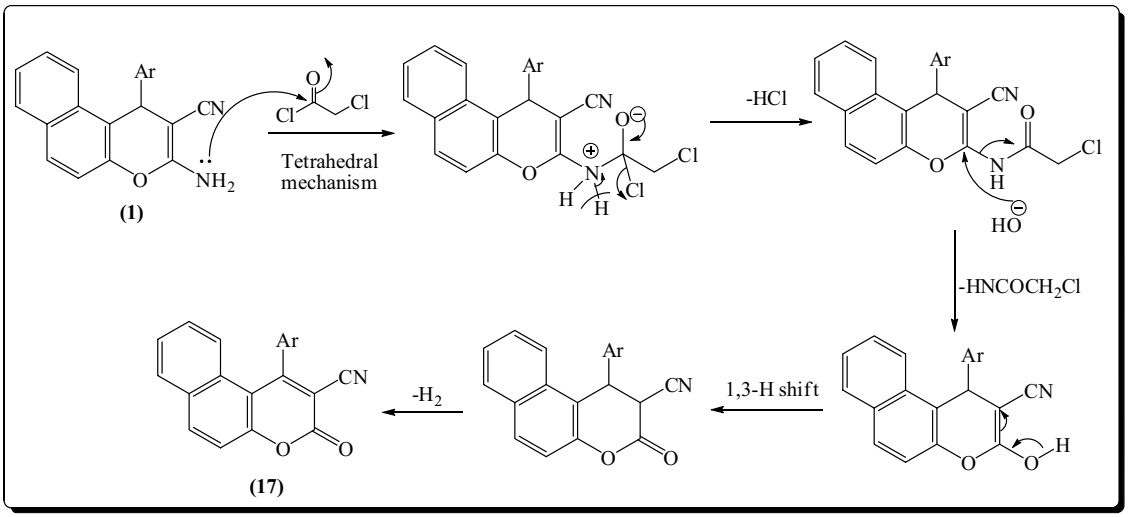
The attempt to chloroacetylate enaminonitrile **1** with chloroacetyl chloride in DMF containing KOH to get the corresponding chloroacetamido derivative resulted in an interesting heterocyclic transformation from benzo[*f*] chromene **1** to benzo[*f*]coumarin **17** which was formerly synthesized [39] from oxidation of the enaminonitrile **1** using I_2O_5 (Scheme 6). Good evidence of the formation of the benzo[*f*]coumarin structure is obtained from IR spectrum of **17** which conserved the absorption band for $\upsilon_{C\equiv N}$ at 2227 cm^{-1} with the appearance of new band at 1738 cm^{-1} due to CO group of coumarin ring. The assigned structure **17** was also confirmed by the ^{1}H-NMR ($CDCl_3$) spectrum which displayed only a multiplet at 7.12–8.21 ppm for 10H attributed to aromatic protons with the absence of the signals of NH, C_4-H protons.

A possible mechanism for the formation of benzo[*f*] coumarin **17** from benzo[*f*]chromene **1** is shown in the Scheme 7.

On the other hand, condensation of enaminonitrile **1** with *p*-nitrobenzaldehyde in glacial acetic acid furnished the corresponding Schiff base **18** (Scheme 6). IR spectrum of **18** exhibited the absence of absorption band of NH_2 group and retained the absorption bands at (ν, cm^{-1}): 2212 (CN) and 1643(C=N).^{1}H NMR ($CDCl_3$) spectrum of **18** showed two singlet at 6.20 and 6.19 ppm (1H, HC_4-pyran and HC_2-pyran, due to 1,3-H shift), two singlets for at N=CH at 9.09, 9.37 ppm due to existence of **18**, in solution in (*Z*) and (*E*) isomers in a ratio of 59.2%: 40.8%, respectively.

In vitro anti-proliferative activity

The in vitro anti-proliferative activity of the newly synthesized compounds was assessed against human liver (HepG2) and breast (MCF7) cancer cell lines in addition to normal fibroblasts (WI-38) and compared to the activity of doxorubicin as a standard drug (Table 1). The data showed that



Scheme 7 A plausible mechanism for the synthesis of compound **17**

Table 1 Influence of the newly synthesized compounds on the viability of HepG2, MCF7 and WI-38 cells

Compd. no.	IC_{50} (μM)[a]		
	MCF-7	HePG2	WI-38
2	71.83 ± 4.6	61.45 ± 3.6	62.8 ± 4.5
3	22.97 ± 1.9	13.49 ± 1.2	58.1 ± 4.3
4	3.79 ± 0.3	2.97 ± 0.2	85.02 ± 4.6
5	87.25 ± 5.5	93.95 ± 5.4	66.0 ± 4.7
6	64.87 ± 4.3	91.78 ± 5.0	29.5 ± 2.2
7	79.91 ± 5.0	75.81 ± 4.1	72.4 ± 4.8
9	19.48 ± 1.7	79.47 ± 4.5	49.2 ± 3.9
10	5.64 ± 0.4	4.81 ± 0.3	91.31 ± 5.1
11	22.09 ± 1.7	21.45 ± 1.7	45.23 ± 2.9
12	53.31 ± 4.0	56.04 ± 3.4	39.1 ± 2.9
13	36.23 ± 2.8	25.89 ± 1.9	23.2 ± 1.2
14	41.58 ± 3.3	26.61 ± 2.0	74.9 ± 2.4
15	57.29 ± 4.1	45.73 ± 2.8	24.29 ± 1.8
16	82.62 ± 5.2	100>	42.2 ± 3.3
17	69.16 ± 4.6	47.27 ± 3.0	51.4 ± 4.1
18	74.67 ± 4.8	68.46 ± 3.9	45.7 ± 3.5
DOX	4.17 ± 0.2	4.50 ± 0.3	6.72 ± 0.5

DOX Doxorubicin

[a]IC_{50} ((μM)): 1–10 (very strong), 11–20 (strong), 21–50 (moderate), 51–100 (weak), above 100 (non-cytotoxic)

doxorubicin had an IC_{50} of ~ 4 to 7 μM against all cells investigated with no differentiation between cancer and normal cells. The novel benzo[*f*]chromene-2-carbohydrazide derivative **4** showed promising anti-proliferative activity against cancer cell lines with IC_{50} of ~ 3 to 4 μM which had much higher potent anti-proliferative activity than the activity of doxorubicin. Also, the novel benzo[*f*]quinoline derivative **10** showed approximately equal activity to doxorubicin against cancer cell lines with IC_{50} of ~ 4.5 to 6 μM. Compounds **4** and **10** were safe to the normal fibroblasts with IC_{50} at ~ 68 to 89 μM. Compounds **4** and **10** showed very strong activity, this is due to the presence of NH and 2 NH_2 groups in **4** and two NH_2 groups in **10** which may be added to any unsaturated moiety in DNA or forming hydrogen bonds with either one of the nucleobases of the DNA and causes it damage (Fig. 4). On the other hand, compound **3** exhibited strong cytotoxic activity against HepG2 cell line with IC_{50} (13.49 ± 1.2) μM, while compound **9** exhibited strong cytotoxic activity against MCF7 cell line with IC_{50} (19.48 ± 1.7) μM. Moderate activities toward HePG-2 and MCF-7 cell lines were observed with compounds **11**, **13** and **14**.

Materials and methods

Chemistry

All melting points were measured on a Griffin and George melting-point apparatus (Griffin & Georgy Ltd., Wembley, Middlesex, UK) and are uncorrected. IR spectra were recorded on Pye Unicam SP1200 spectrophotometer (Pye Unicam Ltd., Cambridge, UK) by using the KBr wafer technique. ^{1}H-NMR spectra were determined on a Varian Gemini 300 MHz and 400 MHz on Bruker Avance III using tetramethylsilane as an internal standard (chemical shifts in δ scale), while ^{13}C NMR spectra were run at 100 MHz. EI-MS was measured on a Shimadzu GC–MS (Columbia, MD) operating at 70 eV. Elemental analyses were carried out at the Microanalytical Unit, Faculty of Science, Ain Shams University, using a Perkin-Elmer 2400 CHN elemental analyzer (Waltham, MA), and satisfactory analytical data

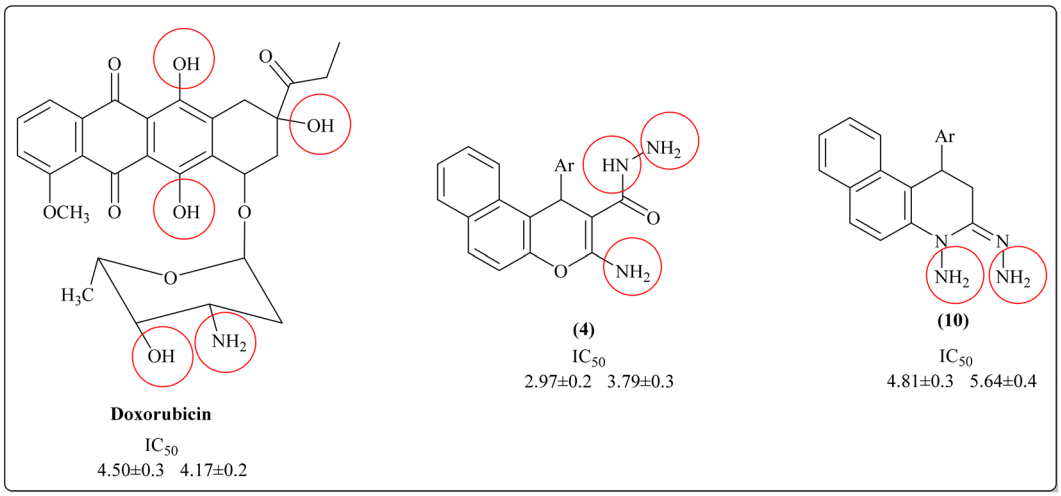


Fig. 4 Structure of doxorubicin and the most potent target compounds **4** and **10**

(±0.4) were obtained for all compounds. The homogeneity of the synthesized compounds was controlled by thin layer chromatography (TLC), using aluminum sheet silica gel F_{254} (Merck). The antitumor activities were performed at Micro analytical Center of Mansoura University, Egypt.

Starting material **1** was synthesized as previously described by us [19]. All other chemicals were obtained from commercial suppliers and were used without any purification. All solvents were distilled from appropriate drying agents before use.

***Synthesis of 10-(2-Chlorophenyl)-9,10-dihydro-8H-benzo[5, 6]chromeno[2,3-b]azet-9-one* (2)** The enaminonitrile **1** (1.66 g, 5 mmol) in formic acid (15 mL) was heated under reflux for 3 h. The excess solvent was distilled off to obtain the crude solid. The obtained solid was filtered off, dried and crystallized from petroleum ether (b.p.: 60–80 °C) to give **2** as white crystals, Yield 1548 mg (93%), mp 206–207 °C. IR spectrum, ν, cm^{-1}: 3439, 1776, 1626. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 4.26 (s, $1H_c$, NH, exchangeable with D_2O), 4.43 (d, $1H_d$, NH, exchangeable with D_2O, $J=6.9$), 5.71 (s, C_4H-pyran), 5.97 (d, C_2H-pyran, $J=6.6$), 6.67–8.03 (m, 10H, Ar–H). ^{13}C-NMR δ, ppm: 39.84, 76.75, 77 (2), 112.70, 113(2), 116 (3), 122 (2), 126.2, 128 (5), 129.1, 130 (5), 131 (4), 133 (4), 148.9, 149.7, 158.5, 159.9. MS (*m/z*, %): 335 ($M^{+\cdot}+2$, 26), 333 (49), 231 (100). Anal. Calcd. for $C_{20}H_{12}ClNO_2$ (333.77): C, 71.97; H, 3.62; N, 4.20. Found: C, 71.87; H, 3.52; N, 4.30.

***Synthesis of 12-(2-Chlorophenyl)-10,12-dihydro-11H-benzo[5, 6]chromeno[2,3-d] pyrimidine-11-one* (3)** A mixture of compound **2** (1.67 g, 5 mmol) and formamide (15 mL) was refluxed for 2 h. The reaction mixture was allowed to cool, and then poured onto ice-cold water. The precipitated solid was filtered off, dried and crystallized from benzene to give **3** as beige crystals, Yield 1278 mg (71%), mp > 300 °C. IR spectrum, ν, cm^{-1}: 3444, 1655. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 5.92, 6.27 (2 s, 1H, HC4-pyran and HC2-pyran; due to 1,3-proton shift, in ratio 42%:58%), 7.05–8.00 (m, 10 H, Ar–H), 8.19 (s, 1H, pyrimidine-H), 12.57 (s, 1H, NH, exchangeable by D_2O). MS (*m/z*, %): 361 ($M^{+\cdot}+1$, 13), 359 ($M^{+\cdot}-1$, 26), 357 (100). Anal. Calcd. for $C_{21}H_{13}ClN_2O_2$ (360.80):C, 69.91; H, 3.63; N, 7.76. Found: C, 69.71; H, 3.56; N, 7.61.

***Synthesis of 3-Amino-1-(2-chlorophenyl)-1H-benzo[f] chromene-2-carbohydrazide* (4)** To a solution of compound **2** (1.67 g, 5 mmol) in ethanol (10 mL), hydrazine hydrate (0.25 mL, 5 mmol) was added dropwise with stirring at room temperature for 30 min. The formed solid was filtered off, dried and crystallized from ethanol to give **4** as white crystals, Yield 1533 mg (84%), mp 167–168 °C. IR spectrum, ν, cm^{-1}: 3442, 3389, 3324, 1650. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 5.90 (s, 1H, HC4-pyran), 6.32 (s, 2H, NH_2, exchangeable by D_2O), 6.90–8.31 (m, 10H, Ar–H), 9.25, 9.45 (2 br.s, 2H, NH_2, exchangeable by D_2O), 11.16 (br.s, 1H, NH, exchangeable by D_2O). MS (*m/z*, %): 366 ($M^{+\cdot}+1$, 26), 332 (100). Anal. Calcd. For $C_{20}H_{16}ClN_3O_2$ (365.82): C, 65.67; H, 4.41; N, 11.49. Found: C, 65.44; H, 4.30; N, 11.53.

***Synthesis of 13-(2-Chlorophenyl)-10,11-dihydro-13H-benzo[5, 6]chromeno[3,2-f][1, 4]oxazepin-12(9H)-one* (5)** A mixture of compound **2** (1.67 g, 5 mmol), and ethanolamine (0.33 mL, 5 mmol) in dioxane (10 mL) was heated under reflux for 2 h. The deposited solid while heating was filtered off, dried, and crystallized from dioxane to give **5** as white crystals, Yield 1319 mg (70%), mp 294–295 °C. IR spectrum, ν, cm^{-1}: 3353, 1688. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 4.5

(br.s, 2H, OCH_2), 4.59 (br.s, 2H, NCH_2), 5.49, 5.56 (2 s, 1H, HC4-pyran and HC2-pyran; due to 1,3-proton shift, in ratio 23%:77%), 7.08–8.11 (m, 10H, Ar–H), 8.86 (br.s, 1H, NH, exchangeable by D_2O). MS (*m/z*, %): 379 ($M^{+\cdot}$+2, 30), 378 ($M^{+\cdot}$+1, 26), 377 ($M^{+\cdot}$, 69), 332 (100). Anal. Calcd. for $C_{22}H_{16}ClNO_3$ (377.82): C, 69.94; H, 4.27; N, 3.71. Found: C, 69.85; H, 4.01; N, 3.65.

***Synthesis of 13-(2-Chlorophenyl)-8,10,11,13-tetrahydrobenzo[5, 6]chromeno[2,3-e][1, 4]diazepin-12(9H)-one* (6)** A mixture of compound **2** (1.67 g, 5 mmol), and ethylenediamine (0.28 mL, 5 mmol) in dioxane (10 mL) was heated under reflux for 2 h. The deposited solid on hot was filtered off, dried, and crystallized from dioxane to give **6** as white crystals, Yield 1146 mg (61%), mp > 300 °C. IR spectrum, ν, cm^{-1}: 3360, 1684. ^1H NMR spectrum, δ, ppm (*J*, Hz): 3.48–3.61 (m, 4H, $2NCH_2$), 5.57, 5.82 (2 s, 1H, H_a, HC4-pyran and H_b, HC2-pyran, due to 1,3-H shift, in ratio 1:1), 6.99–8.16 (m, 10H, Ar–H), 7.3 (s, 1H, NH, exchangeable by D_2O), 8.18 (s, 1H, CONH, exchangeable by D_2O). MS (*m/z*, %): 377 ($M^{+\cdot}$, 86), 315 (100). Anal. Calcd. for $C_{22}H_{17}ClN_2O_2$ (376.84):C, 70.12; H, 4.55; N, 7.43. Found: C, 70.22; H, 4.65; N, 7.33.

***Synthesis of 15-(2-Chlorophenyl)-8,15-dihydrobenzo[b]benzo[5, 6]chromeno[2,3-e][1, 4]diazepin-14(13H)-one* (7)** A mixture of compound **2** (1.67 g, 5 mmol), and *o*-phenylenediamine (0.54 g, 5 mmol) in dioxane (10 mL) was heated under reflux for 6 h. The reaction mixture was concentrated and the solid formed was filtered off, dried, and crystallized from Ethanol/dioxane to give **7** as white crystals, Yield 742 mg (35%), mp 272–274 °C. IR spectrum, ν, cm^{-1}: 3186, 3136, 1724, 1704. ^1H NMR spectrum, δ, ppm (*J*, Hz): 5.68, 5.92 (s, 1H, H_a, HC4-pyran and H_b, HC2-pyran, due to 1,3-H-shift, in ratio 58%:42%), 6.99–7.97 (m, 14H, Ar–H), 8.00 (s, 1H, NH, exchangeable by D_2O), 11.22 (s, 1H, CONH, exchangeable by D_2O). MS (*m/z*, %): 425 ($M^{+\cdot}$, 8), 331 (100). Anal. Calcd. for $C_{26}H_{17}ClN_2O_2$ (424.88): C, 73.50; H, 4.03; N, 6.59. Found: C, 73.40; H, 4.25; N, 6.51.

***Synthesis of 1-(2-Chlorophenyl)-1,2-dihydro-3H-benzo[f]chromen-3-one* (9)** A mixture of the enaminonitrile **1** (1.66 g, 5 mmol), acetic acid (10 mL) and fused sodium acetate (2.00 g) was refluxed for 6 h. The reaction mixture was allowed to cool, poured onto ice-cold water and stirred for 5 min. The precipitated solid was filtered off, dried, and crystallized from ethanol to give **9** as beige crystals, Yield 1370 mg (89%), mp 164–166 °C. IR spectrum, ν, cm^{-1}: 1769. ^1H NMR spectrum, δ, ppm (*J*, Hz): 3.20 (d, 2H, CH_2, $J = 6.0$), 5.45 (t, 1H, C4-pyran, $J = 3.0$), 6.71–7.92 (m, 10H, Ar–H). MS (*m/z*, %): 309 ($M^{+\cdot}$+1, 15), 308 ($M^{+\cdot}$, 11), 231 (100). Anal. Calcd. for $C_{19}H_{13}ClO_2$ (308.76):C, 73.91; H, 4.24. Found: C, 73.98; H, 4.36.

***Synthesis of 1-(2-Chlorophenyl)-3-hydrazono-2,3-dihydrobenzo[f]quinolin-4(1H)-amine* (10)** A mixture of benzo[*f*]chromeneone **8** (1.54 g, 5 mmol) and excess hydrazine hydrate (0.5 mL, 10 mmol) in benzene (10 mL) was stirred at room temperature for 45 min. The deposited solid was filtered off, dried, and crystallized from ethanol to give **10** as white crystals, Yield 1377 mg (82%), mp 234–236 °C. IR spectrum, ν, cm^{-1}: 3327, 3280, 1646, 1627. ^1H NMR spectrum, δ, ppm (*J*, Hz): 2.98–3.00 and 3.08–3.11 (2 d, 2H, CH_2, $J = 6$, 9), 4.07 (s, 2H, NH_2, exchangeable by D_2O), 5.58–5.56 (t, 1H, HC4-pyran, $J = 3.0$), 7.07–8.12 (m, 10H, Ar–H), 9.04, 9.49 (2 s, 2H, NH_2, exchangeable by D_2O). MS (*m/z*, %): 337 ($M^{+\cdot}$+1, 14), 336 ($M^{+\cdot}$, 28), 304 (100). Anal. Calcd. for $C_{19}H_{17}ClN_4$ (336.82): C, 67.75; H, 5.09; N, 16.63. Found: C, 67.65; H, 5.19; N, 16.53.

***Synthesis of 11-(2-Chlorophenyl)-1,2,3,11-tetrahydrobenzo[f]imidazo[1,2-a]quinoline* (11)** A mixture of benzo[*f*]chromeneone **8** (1.54 g, 5 mmol) and ethylenediamine (0.28 mL, 5 mmol) in dioxane (10 mL) was heated under reflux for 45 min. The deposited solid while heating was filtered off, dried, and crystallized from dioxane to give **11** as white crystals, Yield 929 mg (56%), mp 182–183 °C. IR spectrum, ν, cm^{-1}: 3352, 1626. ^1H NMR spectrum, δ, ppm (*J*, Hz): 2.82–2.98 (m, 2H, $NHCH_2$), 3.00–3.10 (m, 3H, $N\underline{CH_2}$+ NH(exchangeable by D_2O)), 5.52 (d, 1H, HC4-pyran, $J = 6.0$), 7.09–8.06 (m, 11H, 10 Ar–H + C=C<u>H</u>). MS (*m/z*, %): 333 ($M^{+\cdot}$+1, 76), 331 ($M^{+\cdot}$− 1, 20), 331 (100). Anal. Calcd. for $C_{21}H_{17}ClN_2$ (332.83): C, 75.78; H, 5.15; N, 8.42. Found: C, 75.84; H, 5.02; N, 8.31.

***Synthesis of 12-(2-Chlorophenyl)-12H-benzo[5, 6]chromeno[2,3-d]pyrimidin-11-amine* (12)** A mixture of the enaminonitrile **1** (1.66 g, 5 mmol) and formamide (15 mL) was heated under reflux for 1 h. The deposited solid formed while reflux was filtered off dried, and then crystallized from dioxane to give **12**, as white crystal, Yield 1579 mg (88%), mp > 300 °C. IR spectrum, ν, cm^{-1}: 3468, 3303, 1642. ^1H NMR spectrum, δ, ppm (*J*, Hz): 6.12 (s, 1H, HC4-pyran), 6.78 (s, 2H, NH_2, exchangeable with D_2O), 7.17–7.99 (m, 10H, 10Ar–H), 8.14 (s, 1H, pyrimidine-H). MS (*m/z*, %): 361 ($M^{+\cdot}$+2, 28), 360 ($M^{+\cdot}$+1, 100). Anal. Calcd. for $C_{21}H_{14}ClN_3O$ (359.81): C, 70.10; H, 3.92; N, 11.68. Found: C, 70.20; H, 3.72; N, 11.75.

***Synthesis of N-Acetyl-N-(12-(2-chlorophenyl)-12H-benzo[5, 6]chromeno[2,3-d]pyrimidine-11-yl)acetamide* (13)** Aminopyrimidine **12** (1.79 g, 5 mol) was heated under reflux in freshly distilled acetic anhydride (10 mL) for 6 h. The excess acetic anhydride was distilled off. The precipitated solid was filtered off, dried and then crystallized from ethanol/dioxane to give **13** as beige crystals, Yield 1484 mg (67%), mp 236–237 °C. IR spectrum, ν, cm^{-1}: 1729, 1704. MS (*m/z*, %): 445

($M^{+\cdot}$+2, 30), 443 ($M^{+\cdot}$, 100). Anal. Calcd. for $C_{25}H_{18}ClN_3O_3$ (443.89):C, 67.65; H, 4.09; N, 9.47. Found: C, 67.54; H, 4.28; N, 9.30.

Synthesis of compounds 14, 15

Method (a): A solution of the enaminonitrile **1** (1.66 g, 5 mmol) in freshly distilled acetic anhydride (20 mL) was heated under reflux for 12 h. The excess solvent was distilled off and the solid formed was fractionally crystallized from benzene to give **14** (1456 mg, 70%) and the insoluble part was crystallized from dioxane to yield **15** (374 mg, 20%).

Method (b): A mixture of the enaminonitrile **1** (1.66 g, 5 mmol) and acetic anhydride (10 mL) in dry pyridine (20 mL) was refluxed for 10 h. The reaction mixture was allowed to cool, poured onto ice-cold water and the formed precipitate was filtered off, washed with water, dried, and then fractionally crystallized from benzene to give **14** (270 mg,13%) and the insoluble fraction was crystallized from dioxane to yield **15** (1570 mg, 84%).

***N-Acetyl-N-(1-(2-chlorophenyl)-2-cyano-1H-benzo[f]chromen-3-yl)acetamide* (14)** white crystals, mp 240–242 °C. IR spectrum, ν, cm^{-1}: 2226, 1742, 1724. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.46 (s, 6H, $2CH_3$), 6.19 (s, 1H, HC4-pyran), 7.08–7.88 (m, 10H, Ar–H). MS (*m/z*, %): 418 ($M^{+\cdot}$+2, 41), 416 ($M^{+\cdot}$, 43), 262 (100). Anal. Calcd. for $C_{24}H_{17}ClN_2O_3$ (416.86): C, 69.15; H, 4.11; N, 6.72. Found: C, 69.05; H, 4.21; N, 6.82.

***12-(2-Chlorophenyl)-9-methyl-10,12-dihydro-11H-benzo[5,6]chromeno[2,3-d]pyrimidin-11-one* (15)** beige crystals, mp > 300 °C. IR spectrum, ν, cm^{-1}: 3442, 1660. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.29 (s, 3H, CH_3), 6.00 (s, 1H, HC4-pyran), 7.10–8.10 (m, 10H, Ar–H), 12.40 (s, H, NH, exchangeable with D_2O). MS (*m/z*, %): 375 ($M^{+\cdot}$+1, 48), 138 (100). Anal. Calcd. for $C_{22}H_{15}ClN_2O_2$ (374.82): C, 70.50; H, 4.03; N, 7.47. Found: C, 70.60; H, 4.13; N, 7.27.

***Synthesis of 12-(2-Chlorophenyl)-11-imino-10-phenyl-8,10,11,12-tetrahydro-9H-benzo[5,6]chromeno[2,3-d]pyrimidine-9-thione* (16)** A mixture of the enaminonitrile **1** (1.66 g, 5 mmol) and excess phenylisothiocyanate (5 mL) was heated under reflux for 6 h. The obtained solid was filtered off, washed with water, dried, and then crystallized from ethanol/dioxane to give **16** as brown crystals, Yield 1260 mg (54%), mp 288–290 °C. IR spectrum, ν, cm^{-1}: 3450, 3380, 1606. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.60 (s, 1H, NH, exchangeable by D_2O), 6.22 (s, 1H, NH, exchangeable by D_2O), 6.52 (s, 1H, $CH_{benzylic}$), 7.02–7.84 (m, 15H, Ar–H), 12.40 (s, H, NH, exchangeable with D_2O). MS (*m/z*, %): 469 ($M^{+\cdot}$+2, 9), 468 ($M^{+\cdot}$+1, 25), 249 (100). Anal. Calcd. for $C_{27}H_{18}ClN_3OS$ (467.97): C, 69.30; H, 3.88; N, 8.98; S, 6.85. Found: C, 69.42; H, 3.67; N, 9.05; S, 6.68.

***Synthesis of 1-(2-Chlorophenyl)-3-oxo-3H-benzo[f]chromene-2-carbonitrile* (17)** To a stirred ice-cooled solution of enaminonitrile **1** (1.66 g, 5 mmol) in DMF (10 mL) containing (0.1 g) of KOH, chloroacetyl chloride (0.52 mL, 5 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was poured onto crushed ice and acidified with acetic acid. The precipitated product was filtered off, washed with water, dried, and crystallized from ethanol to give **17** as yellow crystals, Yield 579 mg (35%), mp 214–216 °C. IR spectrum, ν, cm^{-1}: 2227, 1738. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 7.12–8.21 (m, 10H, Ar–H). MS (*m/z*, %): 333 ($M^{+\cdot}$+2, 46), 331 ($M^{+\cdot}$, 77), 296 (100). Anal. Calcd. for $C_{20}H_{10}ClNO_2$ (331.76): C, 72.41; H, 3.04; N, 4.22. Found: C, 72.31; H, 3.15; N, 4.38.

***Synthesis of 1-(2-Chlorophenyl)-3-(4-nitrobenzylideneamino)-1H-benzo[f]chromene-2-carbonitrile* (18)** A mixture of the enaminonitrile **1** (1.66 g, 5 mmol) and (0.75 g, 5 mmol) of *p*-nitrobenzaldehyde in glacial acetic acid (10 mL) was heated under reflux for 3 h. The deposited product formed after cooling was filtered off, dried and then crystallized from dioxane to give **18** as yellow crystals, Yield 1929 mg (83%), mp > 300 °C. IR spectrum, ν, cm^{-1}: 2212, 1643, 1620. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 6.19, 6.20 (2 s, 1H, HC4-pyran and HC2-pyran; due to 1,3-proton shift), 9.09, 9.37 (2 s, 1H, N=CH due to existence, in solution, of (*Z*) and (*E*) isomers in a ratio of 59.2%: 40.8%), 7.01–8.37 (m, 14 H, Ar–H). MS (*m/z*, %): 467 ($M^{+\cdot}$+2, 47), 465 ($M^{+\cdot}$, 100). Anal. Calcd. for $C_{27}H_{16}ClN_3O_3$ (465.89): C, 69.61; H, 3.46; N, 9.02. Found: C, 69.41; H, 3.56; N, 8.92.

Pharmacological activity

Cytotoxicity assay

The cytotoxic activity of sixteen compounds was tested against two human tumor cell lines, namely: mammary gland (breast) MCF-7 and hepatocellular carcinoma (liver) HePG-2 in addition to normal fibroblasts (WI-38). The cell lines were obtained from the ATCC via the Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Doxorubicin was used as a standard anticancer drug for comparison. The reagents used were RPMI-1640 medium, MTT, DMSO and Doxorubicin (Sigma Co., St. Louis, MO, USA), and Fetal Bovine Serum (GIBCO, Paisley, UK). The different cell lines [40, 41] mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow

tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100 µg/mL streptomycin at 37 °C in a 5% CO_2 incubator. The cell lines were seeded [42] in a 96-well plate at a density of 1.0×10^4 cells/well at 37 °C for 48 h under 5% CO_2 incubator. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µL of MTT solution at 5 mg/mL was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µL was added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, BioTech, Winoosky, VT, USA).

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